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PRINCIPAL INVESTIGATOR: Michael F. Press, M.D.,Ph.D.

CONTRACTING ORGANIZATION: University of Southern California
Los Angeles, California 90033

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13. ABSTRACT *(Maximum 200 words)* This is a molecular epidemiologic case-control study of breast carcinoma *in situ* in Los Angeles County designed to address issues related to the cause and progression of breast CIS by determining epidemiologic risk factors, characterizing selected molecular genetic alterations and prospectively assessing disease progression. The specific aims of the research are 1.) to assess epidemiologic risk factors associated with development of breast CIS, 2.) to determine how frequently specific oncogenes or the p53 tumor suppressor gene are altered in breast CIS, 3.) to investigate potential relationships between various epidemiologic risk factors and somatic genetic alterations and 4.) to assess long-term the association of these factors with disease progression. During the four-year grant period we plan to interview approximately 100 black women and 426 white women (including Hispanics) aged 35-64 years who are diagnosed with breast CIS and who are residents of Los Angeles County, are US-born, and English speaking. The study will utilize 490 black and 490 white control subjects selected by random digit dialing in Los Angeles County who will have been interviewed as part of the Women's CARE Study, a multicentered case-control study of invasive breast cancer being conducted concurrently with this proposed study. We will obtain paraffin-embedded tumor tissue from the pathology laboratories where the patients were diagnosed for analysis of alterations in selected oncogene and tumor suppressor gene expression. Epidemiologic risk factors (reproductive history, lack of participation in physical activity/exercise, positive family history, race, high body mass and exposure to hormones) will be compared with oncogene and tumor suppressor gene expression in breast CIS.

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INTRODUCTION: This is a molecular epidemiologic case-control study of breast carcinoma *in situ* in Los Angeles County designed to address issues related to the cause and progression of breast CIS by determining epidemiologic risk factors, characterizing selected molecular genetic alterations and prospectively assessing disease progression.

Breast carcinoma *in situ* (CIS) is a significant public health problem in Los Angeles County and throughout the United States. Incidence of this disease has been rising steadily, with little epidemiologic or biologic information about breast CIS available. In Los Angeles county, incidence of breast CIS increased from approximately 4 cases per 100,000 women in 1980 to 15 cases per 100,000 women in 1992.

The specific aims of the research are 1.) to assess epidemiologic risk factors associated with development of breast CIS, 2.) to determine how frequently specific oncogenes or the p53 tumor suppressor gene are altered in breast CIS, 3.) to investigate potential relationships between various epidemiologic risk factors and somatic genetic alterations and 4.) to assess long-term the association of these factors with disease progression.

During the four-year grant period we plan to interview approximately 100 black women and 426 white women (including Hispanics) aged 35-64 years who are diagnosed with breast CIS and who are residents of Los Angeles County, are US-born, and English speaking. The study will utilize 490 black and 490 white control subjects selected by random digit dialing in Los Angeles County who will have been interviewed as part of the Women's CARE Study, a multicentered case-control study of invasive breast cancer being conducted concurrently with this proposed study. We will obtain paraffin-embedded tumor tissue from the pathology laboratories where the patients were diagnosed. This case-control study will evaluate reproductive history (early menarche, late menopause, nulliparity or late first birth), lack of participation in physical activity/exercise, positive family history, race (white vs. black), high body mass and exposure to exogenous hormones (oral contraceptives and estrogen or combined estrogen/progestogen replacement therapy) for associations with an increased risk of developing breast CIS. Gene amplification in proto-oncogenes, known to be altered in invasive breast cancer, will be evaluated for changes in pre-invasive breast CIS. The HER-2/*neu* and PRAD1 oncogene will be evaluated for gene amplification by fluorescence *in situ* hybridization and for expression by immunohistochemistry. The p53 tumor suppressor gene, known to be mutated and/or overexpressed in approximately 30% of invasive breast cancers, will be characterized for p53 mutations and overexpression by DNA sequencing and immunohistochemistry. We will determine if any genetic mutations are associated with any of the epidemiologic risk factors investigated. We will also determine if any of the genetic alterations or epidemiologic characteristics are associated with an increased risk of recurrence or progression to invasive disease.

PROGRESS DURING YEAR 01.

EXPERIMENTAL METHODS AND PROCEDURES.

1. Epidemiologic Study Methods.

We are conducting a population-based, case-control study of breast CIS in Los Angeles County using the population-based Cancer Surveillance Program (CSP) at the University of Southern California to identify newly diagnosed patients. Dr. Leslie Bernstein, Co-Principal Investigator, also serves as Scientific Director of the CSP. This investigation is expected to be the largest and most comprehensive epidemiologic and the largest molecular biological characterization of breast CIS yet undertaken. This study will utilize control subjects and methodology from the Women's Contraceptive and Reproductive Experiences (CARE) Study described below.

Timing of the conduct of this proposed study is critical in terms of interviewing women newly diagnosed with breast CIS in Los Angeles County who are eligible for the study. As described below,

we are conducting a case-control study of invasive breast cancer, funded by NICHD, the Women's CARE Study. The Women's CARE Study provide the controls for this epidemiologic study of breast CIS. The breast CIS cases and the Women's CARE Study controls are interviewed concurrently in order to be valid.

Women's Contraceptive and Reproductive Experiences (CARE) Study. The Women's CARE Study is a multicentered case-control study of invasive breast cancer being conducted among women aged 35-64 years in five areas of the US including Los Angeles (PI: Leslie Bernstein, Ph.D.). The Los Angeles Field Center will obtain interviews with 1200 patients with invasive breast cancer and 1200 controls. Half of the patients and half of the controls will be black women; the remainder will be white women (including Hispanic women). Since this study is being conducted concurrently with the study of breast CIS, we utilize the questionnaire that has been designed for the Women's CARE Study and take advantage of the control subjects being interviewed for the Women's CARE Study.

Subject eligibility for the Women's CARE Study is limited to women aged 35-64 years of age who were born in the United States, who are fluent in English, and who have no prior diagnosis of breast CIS or invasive breast cancer.

Controls are selected for the Women's CARE Study by random digit dialing methods. The Centers for Disease Control (CDC) which serves as the Data Coordinating Center for the study has a subcontract with WESTAT for this purpose. Controls are being frequency matched by race and 5-year age category to the case population in Los Angeles. In evaluating the incidence of breast CIS in black and white women in Los Angeles County in 1991 and 1992, we note that the frequency counts in each age group are relatively constant. Since the number of white cases in the Women's CARE Study in each 5-year age category is being held constant in Los Angeles by randomly selecting different proportions in each age group, the distribution of white controls should be optimum for the breast CIS study. For black women, the Women's CARE Study distribution is more heavily weighted toward the older age groups, but because of the fact that 490 controls will be available for the 100 breast CIS cases (see next section below), sufficient controls will be available in each 5-year age category.

The CDC has provided a study management software system (CARETRAC, programmed in Foxpro) that will be utilized for the breast CIS study. This study management system includes automatic generation of letters to physicians and subjects after subject information is entered. It records dates of each step in the process of subject recruitment and has substantial report writing capabilities. It allows for the designation of selection fractions for each 5-year age and race group of women so that one can randomly select patients from among eligible subjects. This selection is done at the time that all eligibility criteria for a given subject have been entered into CARETRAC. CARETRAC maintains records of all contacts with physicians, patients and potential controls.

The Women's CARE Study has implemented strict quality control and data management procedures, all of which are incorporated into this study of breast CIS. These procedures include the selection of a 10% random sample of interviewed subjects for re-interview by the study supervisor. Each questionnaire is visually edited by the interviewer and by the study supervisor. Questionnaire data are entered into the computer using data entry software, SURVEY, provided by the CDC which has programmed checks for out-of-range values and correct skip patterns.

Recruitment of Breast CIS Patients. The selection of cases are restricted to all female cases of breast CIS who are consecutively identified by the CSP and satisfy the following criteria:

1. Age: 35-64.
2. Race: black or white.
3. Birthplace: United States.
4. Language: English speaking.
5. Diagnosis: Breast carcinoma *in situ* (ductal or lobular)
6. Date of Diagnosis: July 1, 1996 and June 30, 1999.
7. Restrictions: No prior diagnosis of breast CIS or invasive breast cancer.

The CSP, the population-based cancer registry for Los Angeles County is operated by the Department of Preventive Medicine at the University of Southern California School of Medicine. As

noted above the Co-PI of this study, Dr. Bernstein, serves as the Scientific Director for the CSP. The CSP began cancer surveillance in Los Angeles County in 1970 and has had nearly complete ascertainment of all incident cancers diagnosed among Los Angeles County residents since 1972. From 1970 until June, 1987, the CSP utilized an active case ascertainment system for identification of cancer patients by deploying medical records technicians to all hospital pathology facilities to search for any reports of cancer. This system was modified somewhat in mid-1987 when the CSP was designated as one of the 10 regional cancer registries for the California Cancer Registry, the statewide registry. At this time state legislation placed the burden of reporting cancer onto the facility or physician's practice where the diagnosis of cancer was made. The CSP has maintained its active case ascertainment component to insure complete reporting and to be able to maintain its repository of pathology reports on all histologically confirmed incident cancers diagnosed among Los Angeles County residents since 1972. In 1992, the CSP was designated as one of the Surveillance, Epidemiology, and End Results (SEER) registries. As a SEER registry, the CSP has instituted follow-up activities on all incident cases diagnosed from 1992 onward to maintain up-to-date information on vital status.

We expect to identify 125 African-American women newly diagnosed with a first primary breast CIS in the three year accrual period and, based on our current response rates in the Women's CARE Study, to interview 100 (80%) of these women. Nearly all of the 125 women will be US born.

We expect to identify 354 white women newly diagnosed with a first primary breast CIS each year who are in the given age range and who are born in the US. We will randomly select half of these women (N = 177 annually) for interview and expect to successfully complete interviews with 142 annually (80%). The random selection process is part of the CARETRAC System currently utilized for the Women's CARE Study and is based on a well-characterized random number generator. We will accrue white women with breast CIS for 3 years and expect to interview a total of 426 white patients in addition to the 100 African-American patients.

Cases for case-control studies are identified by the CSP using a Rapid Case Ascertainment (RCA) procedure. Medical records technicians at the CSP currently visit all pathology laboratories in Los Angeles County on a frequent regular basis, with frequency based on the total number of cancer patients diagnosed at the facility. Each facility is visited at least once a month. The technicians obtain the pathology reports documenting the breast CIS diagnosis, obtain demographic information on the patient (for determination of study eligibility) and express mail or hand deliver the reports to the CSP the same week that the patient is identified. These are then delivered to the Women's CARE Study office across the street from the CSP. Based on this procedure, we expect to identify and interview nearly all breast CIS patients within 3 months of their initial diagnosis.

We utilize the procedures currently used in the Women's CARE Study to obtain physician permission to contact a patient. Currently, we telephone physicians for permission to contact their patients and to obtain updated information regarding the patient's address and any contraindications to contacting the patient. After physician permission is granted, we make initial contact with the patient by letter. This letter advises the patient of the purpose and nature of the study and of our intent to contact her to arrange a personal interview. The patient is then contacted by telephone to seek cooperation and to arrange an appointment for the personal interview. The control contact procedures are identical to those for the case, after the physician has granted us permission to contact the patient.

Batches of eligible controls for the Women's CARE Study are provided to Dr. Bernstein every two weeks by WESTAT following their identification by random digit dialing methods. These methods use a sampling frame of Los Angeles County telephone exchanges that is updated at least every six months.

Data Acquisition. At the time of the interview, informed consent is obtained for the interview, and for patients, authorization to receive biopsy specimen tissue blocks. The epidemiologic interview requires approximately 75 minutes to administer. The questionnaire (see Appendix) contains questions on established and suspected breast cancer risk factors, addressing demographics, pregnancies, menstruation, menopause, surgeries of the breast, ovaries and uterus, hormonal contraception, hormone replacement, other medications, infertility, medical history, mammogram history, physical activity, body size at various ages, cigarette and alcohol consumption histories, first and second degree maternal and

paternal family history of cancer and prenatal exposures. The interviewer utilizes a calendar of life events on which she and the participant record important events, and reproductive and contraceptive history. A photograph album of exogenous hormone preparations is also used to identify specific formulations a woman has used.

All interviewers for the Women's CARE Study undergo a rigorous training period and accreditation by CDC and WESTAT staff before entering the field for the Women's CARE Study. In order to insure that the breast CIS interviewer interviews both breast CIS cases and controls, and to insure that we do not have an unequal balance of patient and control interviews in the Women's CARE Study, the interviewer supported by this study is integrated into the interviewing staff of the Women's CARE Study. The Women's CARE Study currently employs 2.6 FTE interviewers. With the interviewer for the breast CIS patients there are 3.6 FTE interviewers and they are assigned equally to the tasks of interviewing invasive and *in situ* cases as well as controls. This ensures that no bias enters into the assignment for either study.

Retrieving Tissue Specimens: The Women's CARE Study and the CSP have in place mechanisms for the accrual of tissue blocks from surgical pathology laboratories where the biopsy diagnosis was reported since Dr. Bernstein and Dr. Press are collaborating on two other studies including the Women's CARE Study which examines the relationship of prognostic factors and genetic alterations to invasive breast cancer risk factors. Briefly, a request is made by the study staff to the Pathology Retrieval Resource at the CSP for the particular specimens required. This request is accompanied by the patient's signed release of the materials requested. A processing fee is paid to cover the costs of retrieving the materials from the originating laboratory. Tissue specimens are logged into the study database and forwarded to Dr. Press's laboratory for processing.

Data Processing: As noted above, after the questionnaire has been edited by the interviewer and study supervisor, it is key entered into SURVEY, a software package that includes data edits. Any inconsistencies in the data are corrected as they are identified. If necessary, respondents are recontacted to clarify information.

Statistical Analyses: For the case-control study comparisons, we will use standard methods of statistical analysis for unmatched studies. Our approach will be to set up a series of 2 by 2 tables for dichotomous variables and of k by 2 tables for variables with k response levels in the preliminary analyses. Odds ratios and corresponding tests for significance and tests for trends will be computed. We will then utilize unconditional logistic regression to examine multivariate models, evaluating possible confounding and effect modification. All analyses will include adjustment for age in the model. In addition to assessing the magnitude of risk associated with epidemiologic risk factors for breast cancer, we will pay particular attention to whether specific molecular genetic alterations, such as HER-2/*neu* or p53 overexpression modify the effects of these factors on risk of breast CIS. In addition, among breast CIS patients we will examine the associations of individual genetic markers with accepted risk factors for breast cancer (e.g., age, ages at menarche, first term pregnancy and menopause, family history of breast cancer) and other potential risk factors (e.g., physical activity, alcohol consumption). These analyses will utilize standard methods for analysis of categorical data.

Follow-up for Recurrence/Disease Progression: Follow-up activities are performed on an annual basis following completion of the interview. For subsequent years after conclusion of the 4-year funding period, we plan to seek funds to continue the follow-up activities with a goal of completing a 10-year follow-up on these patients. Briefly, breast CIS patients will be actively followed by 1) maintaining contact with them annually to ascertain whether they have experienced any recurrence or progression of their disease, and if so, where treatment occurred and who was the treating physician; 2) maintaining contact with their current oncologist; and 3) by obtaining follow-up information annually from the tumor registry at the hospital at which they were originally diagnosed. Detailed information is obtained on dates of recurrences and diagnosis of more advanced disease. Pathology reports documenting these events is obtained from the pathologist at the laboratories where these diagnoses are made. In addition, medical records are requested from the hospitals and physicians to document these events. As a SEER registry,

the CSP maintains follow-up on all patients with regard to vital status, so that we can annually link our file of interviewed breast CIS patients with this database to determine if any patients have died.

2. Laboratory Methods.

Histopathologic Assessment. Tissue blocks and surgical pathology reports received for each of the cases are reviewed and coded. Microscopic tissue sections (4 microns thick), routinely stained with hematoxylin and eosin, from each tissue block are used for pathologic assessment of each CIS. The cases are classified by the principal investigator as ductal CIS (DCIS) or lobular CIS (LCIS). is sub classified as comedo, cribiform, papillary, micropapillary, solid, or mixed. The presence or absence of other breast pathology including especially ductal hyperplasia and atypical hyperplasia is recorded.

After review of sections from each of the tissue blocks available, the most representative tissue blocks is selected and a total of at least 15 additional sections are cut for the FISH analyses (HER-2/*neu* and cyclin D1), immunohistochemical analyses (ER, PR, HER-2/*neu* , and P53), single-strand conformation polymorphism (SSCP) (p53, exons 2-11) and DNA sequence analysis (p53 exons with mutations by SSCP).

Assessment of Estrogen Receptor and Progesterone Receptor Content. The presence or absence of estrogen receptor (ER) and progesterone receptor (PR) is determined with immunohistochemical assay of paraffin-embedded tissue sections. ER is demonstrated using a monoclonal ER antibody, 1D5, and PR is demonstrated using a monoclonal PR antibody, KD68, in the peroxidase anti-peroxidase technique as described (1). The ER and PR content is recorded as a percentage of positively immunostained cells. Breast CIS cases are characterized as receptor-positive if at least 10% of the CIS tumor cells contain receptor.

Evaluation of Oncogene Expression. Most studies of HER-2/*neu* oncogene expression in breast cancers have used immunohistochemistry as the primary or sole method of analysis. A variety of HER-2/*neu* antibodies have been used in these immunohistochemical studies, however, very little information has been available concerning the antibodies' ability to detect overexpression following tissue processing for paraffin-embedding. Therefore, we evaluated a series of antibodies, reported in the literature or commercially available, to assess their sensitivity and specificity as immunohistochemical reagents and have selected one of the most sensitive antibodies for our studies (2). Immunohistochemistry is used to determine oncogene expression levels in tissue sections. The HER-2/*neu* antibody is a rabbit polyclonal which we have described in previous publications (2, 3, 4, 5).

IMMUNOHISTOCHEMISTRY. Oncoprotein expression in breast CIS (as well as normal and hyperplastic breast epithelium) is assayed by immunohistochemistry using antibodies demonstrated to be sensitive in paraffin-embedded tissues. Our approach to immunohistochemistry involves screening a series of antibodies to the oncoprotein of interest with paraffin-embedded tissues having known expression levels for the oncoprotein. All available antibodies are systematically tested. The antibody with the best performance is selected for routine use in our assays. Our approach to assessing antibodies is described for HER-2/*neu* antibodies in a recent publication (2). This "screening" of antibodies for sensitivity in paraffin-embedded tissue is an important step. Most antibodies function well in frozen tissue, but only some are sensitive in paraffin-embedded tissue. This initial screening step ensures that the most sensitive and specific antibody is used for the assays. Antibodies are already available for each of the oncoproteins described in this proposal. The antibody to be used for HER-2/*neu* has already been selected (2) and results for p53 antibody selection are available.

The immunohistochemical staining method, described elsewhere in detail (2, 3, 4, 5), involves the sequential application of three antibodies to tissue sections as follows: 1.) primary rabbit (or mouse) anti-oncogene antibody, 2.) a secondary or bridging goat anti-rabbit (or -mouse) IgG antiserum (1:75 dilution; Sternberger Monoclonals, Inc.), and 3.) a rabbit (or mouse) peroxidase antiperoxidase antibody (1:75 dilution; Sternberger Monoclonals, Inc.). The primary antibody is incubated overnight at 4°C and the secondary and tertiary antibodies are incubated at room temperature for half of an hour. After treatment with each antibody the tissue sections are washed with phosphate buffered saline. The

immunoprecipitates are identified microscopically after incubation with the chromogen diaminobenzidine. Positive and negative test tissue sections are included with each immunohistochemical procedure as controls. Immunostaining of the various subcellular compartments is interpreted in each of the cell types. The amount of staining is scored in a blinded fashion as negative (no immunostaining), trace positive (weak membrane staining in minority of tumor cells), moderate immunostaining (distinct membrane staining in the majority of cells), or strong immunostaining (intense membrane staining in the majority of cells).

Overexpression of p53 Tumor Suppressor Gene in Breast CIS. p53 is a normal cellular protein which is important in regulating cell growth and metabolism, especially the G1-to-S transition of the cell cycle. However, p53 protein in normal cells of human tissues is present at a low level which is below the level identified by immunohistochemistry. Immunohistochemistry has proven to be a convenient way to distinguish normal expression (negative immunohistochemical staining) from p53 overexpression (positive nuclear immunostaining) in human breast cancers.

IMMUNOHISTOCHEMISTRY. Immunohistochemistry is a very useful method of assessing expression of tumor suppressor genes in tumor specimens. It permits identification of the cell type expressing the tumor suppressor and, if sensitive antibodies and techniques are used, relative expression levels on a cell-by-cell basis. Since immunohistochemistry can be used in paraffin-embedded as well as frozen tissue, it is a very versatile method that is well-suited to a study of breast CIS. We have already tested eleven different p53 antibodies (NCL-p53, CM-1 {Novocastra Labs}, DO-7 {DAKO Corp.}, BP53-12 {BioGenex}, Pab421, Pab1801, Pab 240, Pab246, Pab1620, DO-1 {Oncogene Science}, and CM-10 {Oncor, Inc.}) and selected the most sensitive and specific antibody in paraffin-embedded tissue (Chen and Press, manuscript in preparation). Each antibody has been tested both with and without antigen retrieval techniques (6).

Analysis of p53 Mutations. All of the breast CIS tissue samples are in paraffin-embedded tissue blocks necessitating use of genomic DNA for sequence analysis of p53. Tissue sections mounted on glasss or plastic slides are examined microscopically, DCIS and LCIS (or atypical ductal hyperplasia or other histologic phenotype) are identified, and separated from one another by microdissection.

We have delayed DNA sequence analysis of the p53 gene from our breast CIS cases because we are testing a new DNA sequence technology in collaboration with a group at OncorMed, Inc. This technology is based on the use of microchips with assembled oligonucleotide sequences for hybridization of PCR amplified *p53* from exons 2 - 11. We are testing 250 cancer specimens from our laboratory which have known p53 sequence results previously determined by single-strand conformation polymorphism (SSCP) screening and DNA sequence analysis. These specimens are being characterized the new DNA "chip" technology in a blinded fashion. The results of this approach will be compared with the results of SSCP and DNA sequence analysis to determine the sensitivity and specificity of this new technology and to use the discrepancies to further refine the DNA microchip for p53 analysis. If this technology can be used, we anticipate that this new method will greatly accelerate our ability to analyze *p53* . We also expect the new technology to be more sensitive for detection of all *p53* mutations, since SSCP is variously reported to be able to detect from 70 to 95% of *p53* mutations.

RESULTS AND DISCUSSION.

Epidemiologic Interviews. Our interviews of women in Los Angeles County diagnosed with breast CIS is ahead of our projections in the grant application, partly because support from the SEER Registry Special Studies mechanism permitted us to begin interviewing prior to the start-up date of this USAMRMC grant. We have currently interviewed 566 women and have obtained blood samples from 461 study participants. Permission to study paraffin-embedded tissue blocks was denied from only 25 of the 566 study participants.

Analysis of Breast CIS Tissue Blocks. We have requested paraffin-embedded tissue specimens from 502 cases and to date hospitals have provided paraffin-embedded tissue blocks or

unstained slides from 227 participants. Tissue sections have been reviewed and immunohistochemical assays evaluated from 208 cases and 19 are currently pending. Histologic sections, either prepared from paraffin blocks or provided from referring laboratories, were reviewed from each case to confirm the diagnosis of breast CIS. CIS was not identified in routine hematoxylin-and-eosin stained histologic sections of seven cases. One case contained invasive breast carcinoma and was excluded from the study. In six cases only hematoxylin-and-eosin stained histologic sections were provided; no unstained sections were provided for our subsequent analyses. The breast CIS cases were histologically subclassified as comedocarcinoma (27%), solid (22%), micropapillary (14%), cribriform (13%), papillary (8%), LCIS (6%) and mixed (8%) CIS types. A small number of cases (3%) were considered to show intraductal hyperplasia but not carcinoma. Additional sections were used for the assessment of estrogen receptor, progesterone receptor, HER-2/*neu* oncoprotein and p53 tumor suppressor in the breast CISs.

Estrogen Receptor. The estrogen receptor (ER) content of 120 CIS cases was assessed by immunohistochemistry. 82 CIS cases were ER-positive (68%) and 38 were ER-poor (32%). An additional 88 breast CIS cases have been immunostained for ER and their interpretation is not yet complete.

Progesterone Receptor. The progesterone receptor (PR) content of 120 CIS cases was assessed by immunohistochemistry. 95 CIS cases were PR-positive (79%) and 25 were PR-poor (21%). An additional 88 breast CIS cases have been immunostained for PR and their interpretation is not yet complete.

HER-2/*neu* Oncoprotein. The HER-2/*neu* expression level of 120 CIS cases was assessed by immunohistochemistry. Overexpression of HER-2/*neu* oncoprotein was observed in 46 cases (38%) and low expression was observed in 74 cases (62%). An additional 88 breast CIS cases have been immunostained for HER-2/*neu* and their interpretation is not yet complete.

p53 Tumor Suppressor Protein. p53 tumor suppressor expression was evaluated in 120 cases by immunohistochemistry and 36 cases showed overexpression (30%) while 84 cases (70%) showed no overexpression. An additional 88 breast CIS cases have been immunostained for p53 and their interpretation is not yet complete.

Recommendations in relation to the Statement of Work outlined in the proposal. No change in the original "Statement of Work" is requested at this time. At a later date, if data from preliminary studies warrant a change, the methods that we use to analyze *p53* gene sequence may be changed. In addition, since the primary focus of our study is breast CIS and not other pre-neoplastic histopathologies we are finding that atypical hyperplasias and other non-CIS pathologies are represented in only a limited number of the breast biopsies. The original "Statement of Work" is as follows:

STATEMENT OF WORK.

Technical Objectives:

Task 1. Identify and interview 100 African American and 426 white women with breast CIS in Los Angeles County during the first three years of the grant period. A previously tested epidemiologic questionaire from the Women's CARE Study will be used to interview women entered in this CIS study.

Task 2. Indentify and interview 490 African American and 490 white control women in Los Angeles County during the first three years of the grant period. These control women will be identified from the controls entered in the Women's CARE Study and interviewed as part of that study.

Task 3. Use an epidemiologic interview instrument to determine reproductive history including menarche, menopause, and pregnancy history, participation in physical activity / exercise, family history of breast cancer, race (white vs. black), body mass, and exposure to exogenous hormones (oral contraceptives and estrogen or combined estrogen/progestogen replacement therapy).

Task 4. Tissue blocks and slides will be obtained from the hospital laboratory where the diagnosis of breast CIS was made. The histopathology of each case will be reviewed and characterized.

Task 5. Gene amplification and expression of HER-2/*neu* and cyclin D1 will be evaluated in CIS, atypical hyperplasia, breast duct proliferative epithelium and normal epithelium using fluorescence *in situ* hybridization and immunohistochemistry.

Task 6. Alterations in p53 expression and *p53* gene will be assessed in breast CIS, atypical hyperplasia, breast duct proliferative epithelium and normal epithelium using immunohistochemistry and a combination of SSCP and DNA sequencing.

Task 7. The frequency of alterations in the above oncogenes and p53 tumor suppressor gene will be compared with each other and with the epidemiologic data to assess the frequency of various associations. For example, how often is a history of birth control pill use associated with alterations of any of these genes in breast CIS cells?

Task 8. Use continued followup through the USC Cancer Surveillance Program and through annual contacts with patients and their physicians to determine how frequently women in the study develop recurrent or invasive breast cancer and assess how often these events are associated with particular genetic alterations in breast CIS.

CONCLUSIONS: The preliminary results obtained to date confirmed that ER, PR, p53 and HER-2/*neu* proteins are present in varying proportions in breast carcinoma *in situ* . The pilot study of *p53* mutations in breast carcinoma *in situ* showed that *p53* mutations and p53 overexpression were relatively common in intraductal breast carcinomas and are not observed in adjacent normal breast lobules or ducts. There was a highly significant correlation between *p53* mutations and p53 overexpression in breast CIS. Comparison of epidemiologic risk factors with alterations in ER, PR, HER-2/*neu* , p53 will be performed at the conclusion of the laboratory analyses.

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